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Data Paper

Next release of the European Marine Omics Biodiversity Observation Network (EMO BON) shotgun metagenomic data from water and sediment samples (Release 2)

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Abstract

The European Marine Omics Biodiversity Observation Network (EMO BON) is a long-term genomic observatory run by the European Research Infrastructure European Marine Biological Resource Centre (EMBRC). It was established in 2021 to support the challenges of biodiversity observation and unsystematic management of biodiversity data in the European seas. EMO BON introduced and coordinated the systematic and harmonised observation of biodiversity amongst more than fourteen marine stations in the European coastline. Here, we report the next release (Release 2) of shotgun metagenomic data from seawater and sediment microbial communities.

Keywords

metagenomics, marine biodiversity, ocean observation, water column, soft substrate, microorganisms

Introduction

The advancement of marine biological observation needs to be built on long-term initiatives, able to produce data tackling not only the spatial, but also the temporal component of the dynamic biodiversity. The coordination across different entities and countries has always presented a challenge and a prerequisite for expanded observation capacity in space and time. Research Infrastructures (RIs), as part of the pan-European

scientific landscape, can play a substantial role in this by adding value for their members in providing services and resources, such as management and coordination of marine biological observation.

The EMBRC RI consolidated the marine genomics experience in its community and, therefore, established and coordinated the EMO BON network (Santi et al. 2023). The network widens the distribution of the observatories along the entire European coastline and includes a unique and broad coverage of habitats and longitudes, from the Arctic to the Red Sea. The different observatory stations are coordinated at the RI-level and act as nodes of one integrated network. In 2025, EMO BON is running its fifth year in operation, all the while sample collection and processing, data generation and management and network coordination progress continuously. The aspiration is to maintain the network for at least 10 years of systematic biodiversity observation.

Value of the dataset

This work is the second set of genomic datasets of the EMO BON long-term observation network. It follows up from the first dataset that was realised in this journal in March 2025 (Pavloudi et al. 2025) and will be followed by future releases. All data releases are accompanied by a data publication describing the (meta)data and their online location and allowing for maximum data re-usability and openness, exhibiting the value and potential impact in biodiversity observation research. This release includes raw shotgun metagenomic sequencing data from the seawater and the marine sediment collected between October and December 2021 from 13 observatories, across the European coast and in the Red Sea. The raw sequence data are deposited in and accessible through the European Nucleotide Archive (ENA) (O'Cathail et al. 2024), together with metadata associated with the sampling event, sample preparation, processing and sequencing procedures and a diverse set of measured environmental variables available, such as temperature, salinity and nutrient concentrations in the associated BioSamples (Courtot et al. 2021). Follow-up publications related to the EMO BON macro- and meio-benthos metabarcoding data are upcoming. In addition, it connects with the recent data release from the marine genetic monitoring programme ARMS-MBON (Autonomous Reef Monitoring Structure Marine Biodiversity Observation Network) (Daraghmeh et al. 2025) that was merged into EMO BON and the ARMS data releases that are in preparation (Pagnier et al. 2025).

The present dataset is part of a larger biodiversity time series dataset that is progressively being released in ENA and published in this journal. The impact of the whole dataset is predicted to appear after at least 5 years of data releases, as the value increases with the extension of the data's temporal range. The existence of a marine biodiversity dataset of this depth and quality, spanning at least 5 years and multiple coastal areas in Europe is expected to have a broad appeal, hence increasing the data users and applications.

In addition, this dataset aligns with the mission of the Ocean Biodiversity Information System (OBIS), to facilitate free and open access to biodiversity data and information on marine life. Data products generated after the bioinformatics analyses of this dataset will

include taxonomical and functional information and will be submitted in OBIS and in the Global Biodiversity Information Facility (GBIF), using the DNA extension of the Darwin Core standards. Following the data availability in OBIS and GBIF, the data products will be incorporated in the European Marine Observation and Data Network (EMODnet) and the European Digital Twin of the Ocean (EU DTO). This FAIR data flow into the EU DTO will allow, on one hand, to fill gaps in the current knowledge on biodiversity of the world's oceans and, on the other hand, to model and simulate the 'what if' scenarios, further advancing ocean knowledge and providing information for evidence-based policy-making.

The potential arising from the incorporation of genomic methodologies into marine observation is enormous. Out of many arguments outlining the added value of including genomics to the study of biodiversity in scientific publications (for example, Cordier et al. (2020), Pawlowski et al. (2022), Theissinger et al. (2023)), the most relevant and influential to the EMO BON initiative are the ability to early detect non-indigenous species, the identification of cryptic species and early life stages (e.g. larvae), the interaction amongst species, the study of evolutionary ecology and events shaping species diversity, the access to communities difficult to identify morphologically such as microorganisms and meiofauna, the exploration and explanation of biodiversity patterns across time and space including different communities, from microorganisms to macrobenthos and the response of biodiversity to ecosystem changes (for example, climate change and pollution). Furthermore, the metagenomic analyses of samples allow us to access data and, subsequently, information on function. This leads to additional scientific directions like bioprospecting and biotechnology research, community functioning across time and space and connection of communities functioning to ecosystem changes.

In this dataset, each material sample is represented by identical technical replicates, two of which are processed and sequenced using the exact same methodologies and workflow. The duplicate data can increase the quality of the whole sequencing dataset by allowing the assessment of the processing and sequencing procedures and providing an additional level of quality assurance.

The field of DNA-based environmental research is rapidly evolving and, thus, anticipating the future need for biodiversity re-assessments, another two technical replicates for each material sample are stored in the long-term. The long-term stored replicates can be processed in the future using new technologies or targeted scientific questions. Using the long-term stored samples will open a window in time and allow potential re-sequencing or usage of other omics technologies and combining with the data produced in the present.

Methods

The sampling, sample and data processing methodologies are described in the first EMO BON dataset publication (Pavloudi et al. 2025). The procedures are also briefly described in the following subsections.

Sampling

Seawater sampling was performed by following the Standard Operating Procedures (SOPs) included in the EMO BON Handbook (Santi et al. 2021) and in particular "WaSOP 1 (basic)". Subsurface seawater was collected using Niskin bottles, pre-filtered using 200 µm mesh to exclude particles > 200 µm. The pre-filtered seawater was sequentially filtered through 3 µm and 0.2 µm polycarbonate filter membranes; this produced two seawater samples including particles of different size fractions: 3-200 µm and 0.2-3 µm. Each of the membranes was subsequently cut into two equal pieces using a sterile scalpel; each cut membrane was considered to represent one technical replicate. The sequential filtration took place two separate times producing 2 X the 3-200 µm and 0.2-3 µm membranes, which were then cut into two pieces of equal size, finally generating four technical replicates [First sequential filtration: 3-200 µm membrane cut in half to generate 3-200 µm replicates (1) and (2); and 0.2-3 µm membrane cut in half to generate 0.2-3 µm replicates (1) and (2). Second sequential filtration: 3-200 µm membrane cut in half to generate 3-200 µm replicates (3) and (4); and 0.2-3 µm membrane cut in half to generate 0.2-3 µm replicates (3) and (4)]. Replicates (1) and (2) were preserved in individual tubes using the DNA/RNA shield preservative (Zymo Research) and stored at -80^{o}C until shipment to the sequencing facility. Replicates (3) and (4) were preserved in cryotubes without the addition of DNA/RNA Shield and stored at -80^{o}C for long-term storage.

Sediment was collected by following the Standard Operating Procedures (SOPs) included in the EMO BON Handbook (Santi et al. 2021). In particular, "SoSOP 1 (intertidal sediments)" was followed by the observatories NRMCB and RFormosa, "SoSOP 2 (coastal sediments by diving)" was followed by the observatories ROSKOGO and OOB, "SoSOP 3 (coastal sediments by research vessel)" was followed by the observatory BPNS. In all SOPs, sediment was sampled (or subsampled from the sediment grab in SoSOP 3) using sediment corers. The top 5 cm were sliced, gently homogenised and subsamples were placed in individual tubes to represent four technical replicates. Similarly to the seawater approach, replicates (1) and (2) were preserved in DNA/RNA Shield and stored at -80^{o}C until shipment to the sequencing facility; replicates (3) and (4) were preserved in cryotubes without the addition of DNA/RNA Shield and stored at -80^{o}C for long-term storage.

Geographic range

EMO BON observatories are operating in each of the EMBRC member countries in the EU. This dataset includes data from Norway, Belgium, UK (former EMBRC member), France, Spain, Portugal, Italy and Israel. The geographic range of the dataset includes 14

locations across eight marine ecoregions, based on the Marine Ecoregions of the World (MEOW) (Spalding et al. 2007) (Table 1, Fig. 1). The locality of the observatories is also described using standardised georeferenced terms from the Marine Regions (Flanders Marine Institute 2025), from the broader (ocean/sea) to the regional and the local level (Table 1, Fig. 1). This dataset includes data from the observatory IUIEilat located in the Gulf of Eilat, Indian Ocean, which falls outside the primary European focus of this study.

Table 1.

Coordinates, locality, sampling type (seawater and/or sediment) and number of samples for the operational observatories sampling sites.

Observatory	Coordinates	Ocean/Sea	Region	Location	Marine Ecoregion of the World (MEOW)	Seawater sampling	Sediment sampling	Total Number of samples in this batch	Number of successfully sequenced samples
AAOT	45.31417N; 12.508333E	Mediterranean Sea - Eastern Basin	Adriatic Sea	Gulf of Venice	Adriatic Sea	Yes		8	8
BPNS	51.43333N; 2.808331E	North Atlantic Ocean	North Sea	Belgian part of the North Sea	North Sea	Yes	Yes	12	12
EMT21	42.20194N; -8.798500W	Atlantic Ocean	North Atlantic Ocean	Vigo Seamount	South European Atlantic Shelf	Yes		8	8
ESC68N	68.92589N; 17.125619E	Arctic Ocean	Norwegian Sea	Norwegian part of the Norwegian Sea	Northern Norway and Finnmark	Yes		8	2
IUIEilat	29.50000N; 34.916667E	Indian Ocean	Gulf of Eilat	Gulf of Eilat	Northern and Central Red Sea	Yes		8	8
NRMCB	40.80014N; 14.250000E	Mediterranean Sea - Western Basin	Tyrrhenian Sea	Naples Gulf	Western Mediterranean	Yes	Yes	8	12
OOB	42.489N; 3.143E	Mediterranean Sea - Western Basin	Gulf of Lion	Bay of Banyuls-sur-Mer	Western Mediterranean		Yes	4	2

Observatory	Coordinates	Ocean/Sea	Region	Location	Marine Ecoregion of the World (MEOW)	Seawater sampling	Sediment sampling	Total Number of samples in this batch	Number of successfully sequenced samples
OSD74	41.14653N; -8.666639W	Atlantic Ocean	North Atlantic Ocean	Porto Valley	South European Atlantic Shelf	Yes		8	8
PiEGetxo	43.33858N; -3.014639W	North Atlantic Ocean	Bay of Biscay	Abra de Bilbao	South European Atlantic Shelf	Yes		8	8
RFormosa	37.00564N; -7.969250W	Atlantic Ocean	North Atlantic Ocean	Ria Formosa	South European Atlantic Shelf	Yes	Yes	12	12
ROSKOGO	48.70833N; -3.866000W	North Atlantic Ocean	English Channel	French part of the English Channel	Celtic Seas		Yes	4	4
ROSKOGO	48.77167N; -3.968333W	North Atlantic Ocean	English Channel	French part of the English Channel	Celtic Seas	Yes		8	8
VB	43.68300N; 7.317000E	Mediterranean Sea - Western Basin	Villefranche Bay	Villefranche Bay - Point B	Western Mediterranean	Yes		8	8
MBAL4	50.25N; -4.217W	North Atlantic Ocean	English Channel	Western Channel	Celtic Seas	Yes		8	8

Temporal range

This dataset includes samples collected from October to December 2021.

Sample processing

DNA extraction, library preparation and sequencing

DNA extraction, library preparation and sequencing were collectively performed at the Genoscope French National Sequencing Centre and are described in detail in the first EMO BON dataset publication (Pavloudi et al. 2025). Brief description of sample processing are included here. The water column samples were extracted according to

Alberti et al. (2017): manual cell disruption by cryogenic grinding of membrane filters followed by chemical lysis and nucleic acid purification using NucleoSpin RNA Kits, combined with the NucleoSpin RNA/DNA buffer set (Macherey-Nagel, Düren, Germany). The sediment samples were extracted using the DNeasy PowerSoil Pro Kit (Qiagen) with slight modifications.

Fragments of ~ 350 bp were obtained by sonication. After freebarcodes adapters were added, ligation products were purified using beads and Illumina specific adapters were added by PCR amplifications (2 PCR reactions, 14 cycles). Libraries were quantified and their size profiles analysed prior to sequencing.

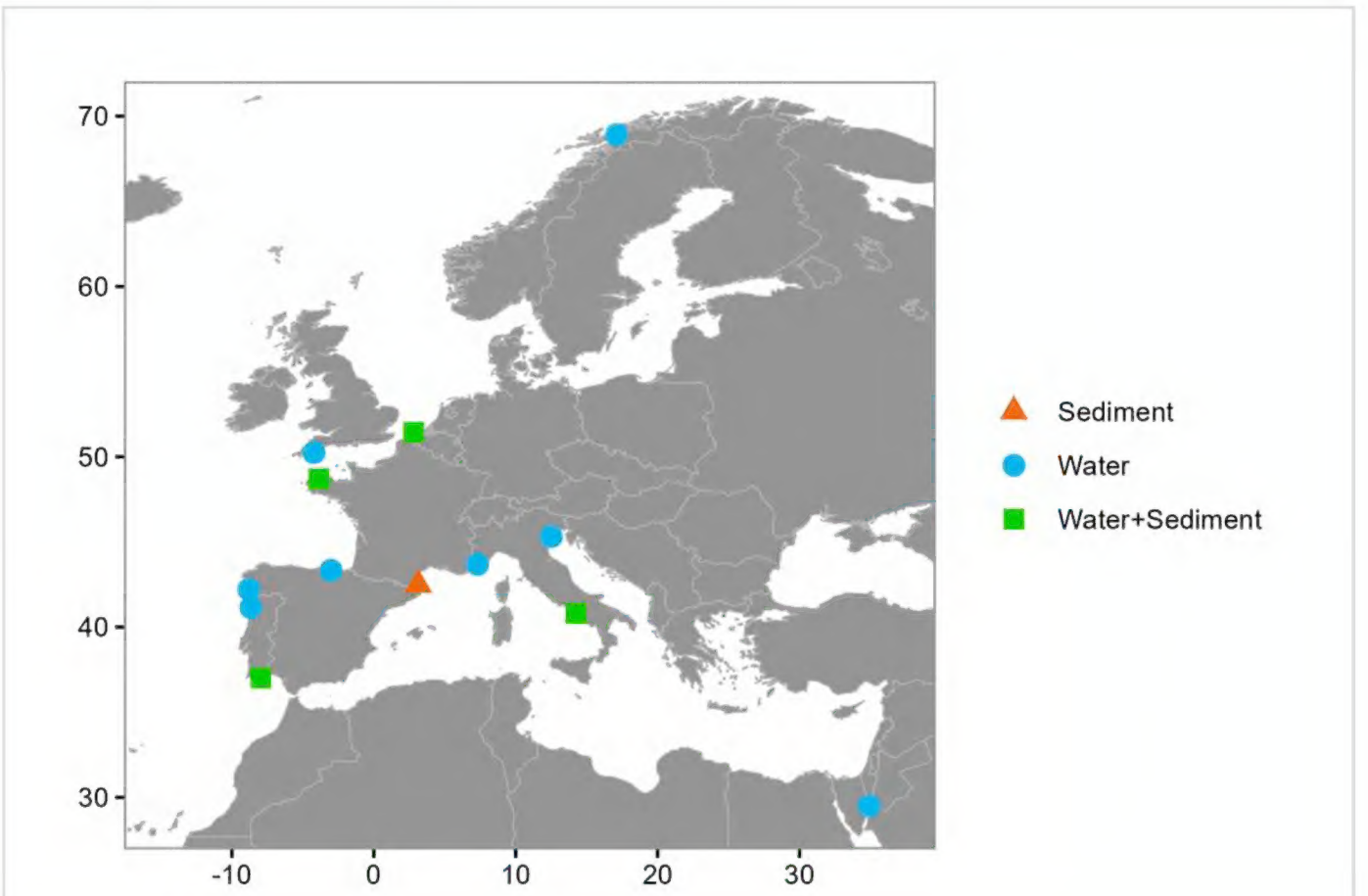

Figure 1. doi

Map of EMO BON observatory sampling sites for sediment (triangle ▲), seawater (circle ●) and both seawater and sediment (square ■), included in this dataset. Observatory IUIEilat located in the Gulf of Eilat, Indian Ocean, falls outside the primary European focus of this study.

Sequencing was performed using 151-bp pairwise read chemistry on an Illumina NovaSeq6000 sequencer, using S4 Flowcells (Illumina, San Diego, CA, USA). A minimum of 40,000 million useful paired-end reads were obtained per sample. Short Illumina reads were bioinformatically post-processed *sensu* Alberti et al. (2017) to filter out low-quality data. Finally, read pairs mapping to the phage phiX genome were identified and discarded using SOAP aligner (Li et al. (2008), default parameters) and the Enterobacteria phage PhiX174 reference sequence (GenBank: NC_001422.1)

Biodiversity scope

Target

Microbial Prokaryotic and Eukaryotic biodiversity in the marine environment.

Taxonomic range

Archaea, Bacteria, Eukaryota

Data Resources

Details for the samples can be found in Suppl. material 1. All the raw sequence files of this study were submitted to ENA (O'Cathail et al. 2024) with the umbrella study accession number PRJEB51688. The accession numbers of the component projects under the umbrella study are PRJEB51656, PRJEB51652, PRJEB51653, PRJEB51657, PRJEB50566, PRJEB51664, PRJEB51662, PRJEB51654, PRJEB51660, PRJEB51661, PRJEB51658, PRJEB51665 and PRJEB51659. All sampling events and environmental data, linked to the respective accession numbers, are also available to browse and download from EMO BON's data landing page.

Resource 1

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Conflicts of interest

The authors have declared that no competing interests exist.

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Supplementary material

Suppl. material 1: ENA accession numbers doi

Authors: Christina Pavloudi
Data type: metadata
Brief description: ENA accession numbers (sample, experiment, run, project and umbrella project) for the EMO BON shotgun metagenomics data release from seawater and sediment samples (batch_2, second release).
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